



Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Lymphocytic Choriomeningitis Virus— A Hazard in Rodent Animal Colonies

In April 2005, a woman died of a stroke and after a thorough work-up, her organs were donated to four recipients. In May, state authorities notified the CDC that three of the transplant recipients had died, and the fourth was ill. Extensive testing revealed that lymphocytic choriomeningitis virus (LCMV) was present in the transplanted organs. Review of the organ donor’s history indicated that she had a new pet hamster, and the hamster was positive for LCMV infection. Although the donor had a subclinical (asymptomatic) infection, the immunosuppressed organ recipients developed fatal or serious infections from the infected organs (CDC, 2005). To prevent disease transmission from pet rodents, CDC has posted advice at: www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lcmv/owners.htm.

Wild, or house, mice are a far more common source of LCMV. LCMV is a rodent-borne Arenavirus, and about 5% of adults living in urban populations have antibodies indicating previous exposure

to LCMV (CDC, 2005). Subclinical LCMV infection in a healthy adult may go unnoticed, but the virus may also produce a flu-like illness, or aseptic meningitis. LCMV is not spread from person to person, except that it may spread vertically from a pregnant woman to a developing fetus. Exposures during the first or second trimester are a serious risk to the fetus (CDC, 2005). To protect the general public from the risk of contracting LCMV from wild rodents, the CDC has posted practical guidelines on the Web and provided detailed information in the publication *Interim Guidance for Minimizing Risk for Human Lymphocytic Choriomeningitis Virus Infection Associated with Rodents* (CDC, 2005).

Case Study 1

In the occupational setting, animal facilities are carefully designed to prevent entry by wild mice and colonies are routinely screened for LCMV. In addition to the zoonotic risk, infection with LCMV can affect a wide range of research results (National Research Council, 1991). The following case study describes the chain of events that lead to an LCMV outbreak at a research institute in 1989 (Dykewicz et al., 1992).

- In 1964, a cancer research institute developed a proprietary cancer cell line. This institute routinely injected the cell line into rodents to induce tumors in order to study metastasis.
- In 1988, the institute began replacing the hamster animal model with nude mice.
- A lapse in the routine serological monitoring of rodent colony health occurred between August 1988 and March 1989. When the monitoring resumed, the oldest sentinel hamsters were positive for LCMV.

- In May 1989, an animal care worker who had never worked with hamsters was hospitalized with aseptic meningitis. With the information that the sentinel animals had LCMV antibodies, the institute's management requested testing to "rule out" LCMV. The hospitalized staff member was diagnosed with LCMV.
- How could this infection have occurred? The proprietary cell line was tested, and it was positive for LCMV. Other staff members began reporting a range of complaints, so the CDC was alerted and an investigation followed.

Results of CDC Investigation

Eighty-two out of 90 staff members consented to serological monitoring for LCMV antibodies. Seven were positive, indicating previous exposure to LCMV, and one was a "probable" previous infection. The control group included 145 local blood donors with only one sample positive for LCMV (Dykewicz et al., 1992). Frozen aliquots of the proprietary cell line were thawed and it was discovered that aliquots as far back as 1975 were seropositive for LCMV. In addition, 13 out of 70 other cell lines passaged in animals were also positive.

So why did the outbreak occur in 1988, if the cells had been infected for a long time? A review of all the factors the eight seropositive staff members had in common revealed no association with needlesticks or sharps injuries, bites or scratches, rural residence, pets, or noncompliance with the use of personal protective equipment. The facility had a policy of requiring gloves and a surgical mask. Only one fact stood out—the eight seropositive staff members had more contact with nude mice (median, 10 hours per week) compared to seronegative staff (median, 1 hour per week) (Dykewicz et al., 1992).

Nude mice are hairless, lack a thymus, and have an impaired immune system. They can become persistently infected with LCMV and excrete it in high titer; this characteristic is aptly called "viruria." (Dykewicz et al., 1992). LCMV infection of nude mice does not produce symptoms in the mice and cannot be detected by direct serological monitoring since nude mice do not produce an antibody response. Apparently, the 80-fold increase in the use of nude mice resulted in a build-up of the viral reser-

voir in this facility and led to seven infections with two hospitalizations and one "probable" infection.

The article goes on to describe the institute's effort to contact former staff members and the many recipients of various cell lines to inform them of the LCMV infection. If the facility administration had not requested testing of the staff member hospitalized with aseptic meningitis, this outbreak might not have been recognized. Even if an animal colony is considered pathogen-free, the use of cell lines passaged in animals in another facility puts the colony, and the staff, at risk.

Case Study 2

An example similar to the one above was presented at the 2004 ABSA conference (Braun, 2004). In 1998, an animal technician developed a persistent high fever of unknown origin and "worrisome" symptoms. Several months later, three sentinel animals in the room where the technician worked were positive for LCMV infection. All of the other technicians, veterinarians, and investigators tested negative, but the animal technician who was ill tested positive. The source was a human cell line obtained from another institution that had been passaged in nude mice for 10 to 12 years without reports of contamination. Fortunately, that outbreak was limited to a single case.

The research community must understand the importance of compliance with a cell line screening policy, since once a study has been approved, it is very difficult to monitor compliance. When incorporated into training programs, case studies such as the ones described above help researchers understand that noncompliance results in risks to themselves, the animal care staff, and their research results.

Additional LCMV Resources

Today, animal care staff and animal researchers receive training on **zoonotic risks**, including LCMV. For example, the University of California-Irvine web site has the following statement under "rodent health" at: www.rgs.uci.edu/ular/policies/rodentcolonyhealth.htm.

The overwhelming significant feature of LCMV is its zoonotic potential. It can be predicted that T cell-deficient mice will amplify LCMV infection. The polytropic nature of LCMV and its wide host range allow this virus to readily infect transplantable tumors and cell lines, which can serve as a source of contamination for mouse colonies.

- Organotropism—kidney, salivary gland, lymphohematopoietic cells
- A zoonosis
- Interference with research—immunology, oncology, physiology

Well-established procedures for using immunocompetent **sentinel animals** are described at the University of Washington web site at: <http://depts.washington.edu/compmed/rodenthealth/#sentinels>.

Roughly analogous to the “canary in the coal mine,” sentinel rodents monitor the pathogen status of the investigator’s rodents. Every rack in a room is monitored. As you are facing a rack, the cage containing the two sentinels usually resides in the lower right-hand position. Every time an investigator’s rodent cage is changed (usually weekly), about a tablespoon of soiled bedding from that cage is transferred to the sentinel cage. In this way, sentinels are exposed to whatever pathogens may be present in the urine, feces, fur, saliva, dander, etc. from 100% of the cages on the rack. Because of this, investigators must not handle or move sentinels or sentinel cages.

PCR testing for many zoonotic pathogens is also commercially available; this allows direct testing of immunocompromised strains. Charles River Laboratory has posted an explanation of test methods available at: www.criver.com/research_models_and_services/research_animal_diagnostics/LAD_DS_

[MolecularW.pdf](#) and www.criver.com/research_models_and_services/research_animal_diagnostics/LAD_DS_InVivoBiosafetyW.pdf.

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